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Prophylactic Effect of Topical Besifloxacin and Moxifloxacin on the Bacterial Conjunctival Flora Before and After Intraocular Surgery

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ABSTRACT

Aim: To study bacterial conjunctival flora before and after topical moxifloxacin or besifloxacin used as prophylactic agent in intraocular surgeries. **Settings and design:** Prospective randomized study. **Material and methods:** Conjunctival swabs of 100 patients undergoing intraocular surgeries were collected 2 days before the surgery without prior antibiotic use and inoculated on culture media for culture and antibiotic sensitivity tests. Patients were randomized into two groups (50 each). Patients of Group A and Group B received topical moxifloxacin 0.5% and besifloxacin 0.6% eye drop, respectively, to the assigned eye 6 hourly. Postoperatively, antibiotic eye drops were instilled 4 hourly for 10 days and then stopped. Topical anti-inflammatory and steroid drugs were continued for 6 weeks. Conjunctival swabs were repeated from operated eyes 20 and 40 days postoperatively. Statistical analysis was done using Chi-square and McNemar's tests. **Results:** Bacterial growth appeared in 27 cases (most commonly *Staphylococcus epidermidis* 51.85%) - 16 in Group A and 11 in Group B - and none of the isolate showed resistance to the assigned antibiotic. **Conclusions:** The antibacterial efficacy of topical moxifloxacin and besifloxacin in preventing postoperative infections is similar; hence, both may be equally effective for prophylaxis in intraocular surgeries.

Keywords: Conjunctival flora, moxifloxacin, besifloxacin, intraocular surgeries, prophylaxis

The term "normal conjunctival flora" refers to microorganisms that dwell within the eyes of healthy individuals. Predominant isolates recovered from the normal adult conjunctiva are *Staphylococcus epidermidis, Staphylococcus aureus, Streptococcus* nonhemolytic and *Propionibacterium acnes*.

The knowledge of these organisms and their antibiotic sensitivity/resistance provides a better guide in choosing an appropriate antibiotic for prophylaxis of postoperative infections for which topical fluoroquinolones are commonly used. Due to antibiotic resistance, proper selection of antibiotic remains a challenge for ophthalmologists.

The present study was planned to ascertain normal conjunctival flora and its sensitivity/resistance to the new fourth-generation fluoroquinolones viz. moxifloxacin and besifloxacin.

MATERIAL AND METHODS

This prospective randomized study was conducted on 100 eyes of 100 patients who presented at Eye OPD in our institution from February 2014 to August 2015 for treatment of diseases requiring intraocular surgeries. The details of the procedures were explained to all the patients in their language and written consent was obtained. Ethical clearance for the study was granted by the Institutional Ethics Committee.

Patients with hypersensitivity to moxifloxacin and besifloxacin or any of the ingredients in the study medications were excluded from this study. Patients who had used any topical antibiotic drops 3 months prior to culture, taken systemic antibiotics 1 month before and during the study period, neonates, infants, pregnant and lactating females were also excluded.

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Moxifloxacin ophthalmic solution and besifloxacin ophthalmic suspension were used for the study.

After taking first preoperative conjunctival swab from the study eye, patients were randomly divided in two groups, A and B, with 50 patients each. Patients of Group A (Moxifloxacin Group) received topical moxifloxacin 0.5% eye drops and patients of Group B (Besifloxacin Group) received topical besifloxacin 0.6% eye drops to the assigned eye 6 hourly for 2 days preoperatively and continued postoperatively every 4 hours for 10 days, and then stopped. Topical anti-inflammatory and steroid eye drops were started from first postoperative day for 6 weeks. Conjunctival swabs were repeated from the operated eyes 20 and 40 days postoperatively and inoculated on culture media to look for bacterial growth.

Sterile cotton swabs with polypropylene stick were used for obtaining conjunctival swab from the eyes of patients. The swabs were inoculated on Blood Agar and Chocolate Agar and incubated at 37°C overnight. Positive cultures were processed for identification of organisms by studying Gram-staining, colony characteristics and several biochemical tests. Later on, antibiotic sensitivity was assessed for moxifloxacin using the commercially available disc containing 5 μg of the drug. Antibiotic discs of besifloxacin are not available commercially, and the sensitivity was assessed using self-made discs of sterile Whatman No. 41 filter paper of 5 mm diameter and eye drop besifloxacin 0.6%, finally achieving a concentration of 10 μg/disc. Antibiotic sensitivity testing was done by the Standard Disc Diffusion method described by Bauer et al (1966).

The results were analyzed statistically using Chi-square and McNemar's tests.

RESULTS

A total of 100 eyes of 100 patients (62 males and 38 females) were included in the study; majority (68%) were between the ages of 51 and 70 years. Maximum number of patients (85/100) underwent manual small incision cataract surgery (MSICS) with posterior chamber intraocular lens (PCIOL) implantation. Nine patients underwent trabeculectomy and 6 patients had combined trabeculectomy with MSICS with PCIOL implantation. Conjunctival swabs were obtained from 100 eyes (50 in moxifloxacin and 50 in besifloxacin group) and inoculated on bacterial culture media. Twenty-seven swabs yielded growth of bacteria (positive cultures) with no statistically significant difference between males and females as shown in Table 1.

The results of the study indicated higher positive cultures occurring in early summer season and also slightly higher in older patients (more than 50 years of age), although the difference was statistically insignificant. Out of 27 positive cultures, 23 (85.2%) cases were patients with mature and immature age-related cataract, 2 (7.4%) cases were with primary open-angle glaucoma and other 2 (7.4%) with co-existing cataract and glaucoma. The association of various diagnoses with positive conjunctival culture was statistically insignificant (p = 0.893). In our study, the most common organisms isolated were *S. epidermidis* (51.85%), *S. aureus* (29.63%), followed by *Streptococcus pyogenes* (11.11%) and *Corynebacterium xerosis* (7.41%) as shown in Table 2.

The intraocular surgery of the patients showing bacterial growth on culture plates was postponed and the designated antibiotic was continued for 5 days. Conjunctival swabs were obtained after a week of drug-free period and at this time, there was no bacterial growth in any of the 27 patients as the organisms were sensitive and therefore eliminated from the conjunctival sac. The same antibiotics were restarted 2 days before the surgery and rest of the procedure was followed as described for other patients. None of the patients included in the study developed postoperative endophthalmitis. The inhibition of bacterial growth was 100% in both the groups viz. moxifloxacin and besifloxacin and all the laboratory cultures showed no bacterial growth on second and third visits, revealing the sensitivity of organisms to be almost similar to both the test antibiotic eye drops as shown in Tables 3 and 4.

Table 1. Gender-wise Distribution of Positive Cultures among Patients

Gender	Positive culture n (%)	Negative culture n (%)	Total n (%)
Male	16 (25.81)	46 (74.19)	**62 (100)**
Female	11 (28.95)	27 (71.05)	**38 (100)**
Total	**27 (27)**	**73 (73)**	**100 (100)**

χ^2 = 0.118, df = 1, p = 0.731

Table 2. Occurrence and Distribution of Isolated Organisms

Operated eyes	Total No. of eyes	Positive culture	Organisms isolated							
			SE		SA		SP		CX	
			No.	%	No.	%	No.	%	No.	%
RE	56	15	07	46.67	05	33.33	02	13.33	01	6.67
LE	44	12	07	58.33	03	25	01	8.33	01	8.33
Total	**100**	**27**	**14**	**51.85**	**08**	**29.63**	**03**	**11.11**	**02**	**7.41**
$\chi^2 = 0.506$, df = 3, p = 0.918										

SE: *Staphylococcus epidermidis*; SA: *Staphylococcus aureus*; SP: *Streptococcus pyogenes;* CX: *Corynebacterium xerosis;* RE: Right eye; LE: Left eye.

Table 3. Group A: Effect of Topical Moxifloxacin on Bacterial Conjunctival Flora

Before use		After use			
1st Conjunctival swab (Preoperative)		2nd Conjunctival swab (Postoperative Day 20)		3rd Conjunctival swab (Postoperative Day 40)	
Positive	Negative	Positive	Negative	Positive	Negative
16	34	00	50	00	50
McNemar's $\chi^2 = 5.78$, df = 1, p = 0.016					

Table 4. Group B: Effect of Topical Besifloxacin on Bacterial Conjunctival Flora

Before use		After use			
1st Conjunctival swab (Preoperative)		2nd Conjunctival swab (Postoperative Day 20)		3rd Conjunctival swab (Postoperative Day 40)	
Positive	Negative	Positive	Negative	Positive	Negative
11	39	00	50	00	50
McNemar's $\chi^2 = 14.58$, df = 1, p < 0.001					

DISCUSSION

Prevention of infections following intraocular surgeries is one of the areas of maximum concern to all ophthalmic surgeons. This is especially true because recent reports suggested that though the incidence of postoperative endophthalmitis has decreased significantly in present era, the emergence of resistance among bacterial isolates to routinely used prophylactic antibiotics is a matter of great concern for eye specialists world over. In view of the possible role of conjunctival flora in the causation of any postoperative infections following intraocular surgeries along with an emergence of multidrug-resistant organisms, an understanding of the sensitivity of such flora to appropriate antibiotics is of fundamental importance. Certainly, such type of studies might guide the ophthalmologists when using a prophylactic antibiotic before performing a surgery.

Therefore, the present study aims to assess the normal conjunctival flora and possible role of two newer fourth-generation fluoroquinolones (moxifloxacin and besifloxacin) as one of the preventive measures against postoperative infections. The study was done on 100 patients admitted for various intraocular surgeries; majority of them comprised of age-related cataract (85/100), 11 being mature and 74 being immature age-related cataracts. This is because of the fact that cataract continues to be the leading cause of ocular morbidities requiring intraocular surgeries. In the present series, majority of the admitted patients (85 out of 100) underwent MSICS with PCIOL implantation followed by trabeculectomy and combined trabeculectomy with MSICS with PCIOL implantation.

McNatt et al (1978) reported that out of the 184 eye cultures, 112 (60.9%) contained at least one microorganism. Herde et al (1996) reported that out of 686 conjunctival swab cultures, 126 (18.4%) showed bacterial growth. Our study demonstrated only 27 positive bacterial growths out of 100 cases (27%) as shown in Table 1. This variable incidence could be due to variable environmental and individual factors.

In the present study, the bacterial conjunctival flora observed was almost the same in either sex (25.81% in males and 28.95% in females) as shown in Table 1. Rao and Rao (1972) also did not observe any difference in conjunctival bacterial flora of either sex (22.5% in males and 18.2% in females). They also studied the variation of conjunctival bacterial flora in relation to weather and observed a higher rate of positive bacterial cultures during summer season. Our results too indicated higher positive cultures occurring in early summer season, highest being in the month of April. The incidence of positive bacterial culture was observed to be slightly higher in older patients (>50 years of age), although the difference was statistically insignificant. Singer et al (1988) reported similar results in their study.

There was no association of various diagnoses with positive conjunctival culture. de Kaspar et al (2004) also found no relationship between the conjunctival flora and the ocular morbidities (83% positive in eyes undergoing cataract surgery and 77% in those undergoing glaucoma surgery; $p = 0.2246$).

Many researchers have studied the composition of normal conjunctival flora. Nema et al (1964) found coagulase-negative staphylococci as the most common organism isolated from conjunctiva. The other isolated organisms included Diphtheroids, coagulase-positive staphylococci, streptococci, pneumococci, Gram-positive spore bearing bacilli and various Gram-negative coliform bacilli. Gritz et al (1997) isolated *S. epidermidis* in 54.8% and Diptheroids in 9.5% subjects in conjunctival swabs. In our study, we noticed *S. epidermidis* (51.85%) as the most common organism isolated from conjunctiva, followed by *S. aureus* (29.63%), *S. pyogenes* (11.11%) and *C. xerosis* (7.41%) as shown in Table 2.

A number of other studies have revealed *S. epidermidis* to be the most frequently isolated organism from the conjunctival sac. This organism, being a part of normal bacterial flora of conjunctiva remains nonpathogenic among healthy individuals but can cause severe infections in the eye, including endophthalmitis, in altered conditions. Therefore, all the conjunctival organisms, including staphylococci harbored by human conjunctiva, need attention before performing any intraocular surgery in order to prevent any postoperative infection.

O'Brien et al (2007) found that moxifloxacin had potent and rapid bactericidal activity against most of the Gram-positive pathogens causing postoperative endophthalmitis, and had excellent ocular penetration after topical administration. Scoper (2008) also reported that fourth-generation fluoroquinolones (moxifloxacin and gatifloxacin) had increased potency against Gram-positive bacteria compared with third-generation fluoroquinolones (levofloxacin), while maintaining similar potency against Gram-negative bacteria.

In our study, bacterial growth was seen in 16 preoperative conjunctival swabs in moxifloxacin group and all of these isolates were found to be sensitive to moxifloxacin on performing *in vitro* antibiotic sensitivity tests and complete eradication of bacteria, as evidenced by conjunctival swab culture, was obtained after pre- and postoperative use of this antibiotic as depicted in Table 3.

Moshirfar et al (2006) first reported 2 cases of bacterial keratitis-resistant to fourth-generation fluoroquinolones after laser *in situ* keratomileusis (LASIK) and photorefractive keratectomy (PRK). Yin et al (2013) also found that repeated use of topical moxifloxacin after intravitreal injection significantly increased antibiotic resistance of ocular surface flora and recommended not to use prophylactic antibiotics routinely after intravitreal injections. Oldenburg et al (2013) isolated 89 *Pseudomonas aeruginosa* isolates during 2007, 2008 and 2009 in "The Steroids for Corneal Ulcers Trial" (SCUT) and reported an increase in the proportion of resistant isolates to moxifloxacin from 19% in 2007 to 52% in 2009. An increase in resistance to the fourth-generation fluoroquinolones was detected for both methicillin-resistant *S. aureus* (MRSA) and methicillin-sensitive *S. aureus* (MSSA) by Chang et al (2015).

In spite of reports of emergence of resistance against widely used moxifloxacin, no bacterial strain isolated in our study showed resistance to the drug. Similarly, it remained effective in the moxifloxacin receiving group as suggested by negative bacterial cultures taken 20 and 40 days postoperatively. One of the reasons for not detecting resistance to the drug might be attributed to the low prevalence of organisms with resistance to moxifloxacin in the studied population.

The other group of patients, comprising of 50 subjects undergoing various intraocular surgeries, received topical besifloxacin eye drop (0.6%). In May 2009, besifloxacin, a fluoroquinolone, was approved by the Food and Drug Administration (FDA) as a topical agent for the treatment of bacterial conjunctivitis. The results of a study conducted by Haas et al (2010) have confirmed that besifloxacin has potent *in vitro* activity against bacterial isolates including *S. aureus, S. epidermidis* and *S. pneumoniae.* Similar type of antibacterial activity against some of the isolates resistant to other fluoroquinolones was also evident in a study that evaluated the antibacterial spectrum of besifloxacin against as much as 40 Gram-positive and Gram-negative species (Haas et al, 2009).

In our study, the prophylactic potential of besifloxacin was examined *in vitro* against only Gram-positive organisms as no Gram-negative organism could be recovered in the bacterial conjunctival flora of the patients included. All the organisms isolated from Group B patients were sensitive to besifloxacin because no bacterial growth was seen in postoperative microbiological examination as shown in Table 4. Sanders et al (2009) also demonstrated that besifloxacin was significantly more effective than gatifloxacin and moxifloxacin in reducing the number of MRSA in the rabbit cornea 16 hours after infection.

Both the fluoroquinolones under study (moxifloxacin and besifloxacin) seemed to be highly effective in *in vitro* sensitivity test conducted against all the bacterial isolates recovered from the patients undergoing various intraocular surgeries. Further, the *in vivo* use in pre- and postoperative period in the eyes of as much as 100 patients before undertaking intraocular surgeries and postoperatively at Day 20 and Day 40 revealed their effective potential as possible prophylactic agents in ophthalmic surgeries (Table 3 and 4).

CONCLUSION

On comparing the activity against the bacterial isolates, no significant difference was observed and both the antibiotics (moxifloxacin and besifloxacin) showed an effective antibacterial potential. Thus, these antibiotics can be used in ophthalmology as effective antibacterial prophylactic agents among the patients undergoing various intraocular surgeries.

SUGGESTED READING

1. Lactocher-Khorazo D, Seegal BC. Bacteriology of the eye. St Louis: Mosby; 1972. pp. 13-7.
2. Kecik T, Pauk M, Mularczyk H, Marciniak A. Bacterial flora in the conjunctival sac of patients before cataract surgery. Klin Oczna. 1995;97(7-8):252-4.
3. Ueta M, Iida T, Sakamoto M, Sotozono C, Takahashi J, Kojima K, et al. Polyclonality of *Staphylococcus epidermidis* residing on the healthy ocular surface. J Med Microbiol. 2007;56(Pt 1):77-82.
4. Barría von-BF, Chabouty H, Moreno R, Ortiz F, Barría MF. Microbial flora isolated from patient's conjunctiva previous to cataract surgery. Rev Chilena Infectol. 2015;32(2):150-7.
5. Ciulla TA, Starr MB, Masket S. Bacterial endophthalmitis prophylaxis for cataract surgery: an evidence-based update. Ophthalmology. 2002;109(1):13-24.
6. Garg P, Mathur U, Sony P, Tandon R, Morris TW, Comstock TL. Clinical and antibacterial efficacy and safety of besifloxacin ophthalmic suspension compared with moxifloxacin ophthalmic solution. Asia Pac J Ophthalmol (Phila). 2015;4(3):140-5.
7. Bucci FA Jr, Evans RE, Amico LM, Morris TW, Fluet AT, Sanfilippo CM, et al. Antibacterial efficacy of prophylactic besifloxacin 0.6% and moxifloxacin 0.5% in patients undergoing cataract surgery. Clin Ophthalmol. 2015;9:843-52.
8. Bauer AW, Kirby WM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disk method. Am J Clin Pathol. 1966;45(4):493-6.
9. McNatt J, Allen SD, Wilson LA, Dowell VR Jr. Anaerobic flora of the normal human conjunctival sac. Arch Ophthalmol. 1978;96(8):1448-50.
10. Herde J, Tost M, Wilhelms D, Höhne C, Thiele T. Perioperative conjunctival flora. Klin Monbl Augenheilkd. 1996;209(1):13-20.
11. Rao PN, Rao KN. Study of the normal conjunctival flora (bacterial and fungal) and its relations to external ocular infections. Indian J Ophthalmol. 1972;20(4):164-70.
12. Singer TR, Isenberg SJ, Apt L. Conjunctival anaerobic and aerobic bacterial flora in paediatric versus adult subjects. Br J Ophthalmol. 1988;72(6):448-51.
13. de Kaspar HM, Kreidl KO, Singh K, Ta CN. Comparison of preoperative conjunctival bacterial flora in patients undergoing glaucoma or cataract surgery. J Glaucoma. 2004;13(6):507-9.
14. Nema HV, Bal A, Nath K, Shukla BR. Bacterial flora of the trachomatous conjunctiva. Br J Ophthalmol. 1964;48:690-1.
15. Gritz DC, Scott TJ, Sedó SF, Cevallos AV, Margolis TP, Whitcher JP. Ocular flora of patients with AIDS compared with those of HIV-negative patients. Cornea. 1997;16(4):400-5.
16. O'Brien TP, Arshinoff SA, Mah FS. Perspectives on antibiotics for postoperative endophthalmitis prophylaxis: potential role of moxifloxacin. J Cataract Refract Surg. 2007;33(10):1790-800.
17. Scoper SV. Review of third- and fourth-generation fluoroquinolones in ophthalmology: in-vitro and in-vivo efficacy. Adv Ther. 2008;25(10):979-94.
18. Moshirfar M, Mirzaian G, Feiz V, Kang PC. Fourth-generation fluoroquinolone-resistant bacterial keratitis after refractive surgery. J Cataract Refract Surg. 2006;32(3):515-8.
19. Yin VT, Weisbrod DJ, Eng KT, Schwartz C, Kohly R, Mandelcorn E, et al. Antibiotic resistance of ocular surface flora with repeated use of a topical antibiotic after intravitreal injection. JAMA Ophthalmol. 2013; 131(4):456-61.
20. Oldenburg CE, Lalitha P, Srinivasan M, Rajaraman R, Ravindran M, Mascarenhas J, et al. Emerging moxifloxacin

resistance in *Pseudomonas aeruginosa* keratitis isolates in South India. Ophthalmic Epidemiol. 2013;20(3):155-8.

21. Chang VS, Dhaliwal DK, Raju L, Kowalski RP. Antibiotic resistance in the treatment of *Staphylococcus aureus* keratitis: a 20-year review. Cornea. 2015;34(6):698-703.
22. Haas W, Pillar CM, Hesje CK, Sanfilippo CM, Morris TW. Bactericidal activity of besifloxacin against staphylococci, *Streptococcus pneumoniae* and *Haemophilus influenzae*. J Antimicrob Chemother. 2010;65(7):1441-7.
23. Haas W, Pillar CM, Zurenko GE, Lee JC, Brunner LS, Morris TW. Besifloxacin, a novel fluoroquinolone, has broad-spectrum in vitro activity against aerobic and anaerobic bacteria. Antimicrob Agents Chemother. 2009;53(8):3552-60.
24. Sanders ME, Norcross EW, Moore QC 3rd, Shafiee A, Marquart ME. Efficacy of besifloxacin in a rabbit model of methicillin-resistant *Staphylococcus aureus* keratitis. Cornea. 2009;28(9):1055-60.

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J Hypertens. 2014;32(6):1334-41.